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Elucidating Astragaloside IV's Anti-Glioma Action via Network Pharmacology, Molecular Docking, and Experimental Evidence for PI3K/AKT Pathway Inhibition

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Abstract **Objective:** To investigate astragaloside IV's (AS-IV) anti-glioma effects and mechanisms. **Methods:** Potential AS-IV targets were screened using SwissTarget, Super PRED, and PharmMapper databases. Glioma-related targets were identified from GeneCards, OMIM, and TTD. Intersection genes underwent Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. Anti-glioma mechanisms were investigated using A172 and U251 glioma cell lines. Cells were treated with AS-IV at 20, 30, or 50 mg/mL. MTT assessed proliferation. Cell scratch and transwell assays evaluated invasion. Flow cytometry analyzed cell cycle distribution and apoptosis. Western blot measured expression of cell cycle, apoptosis, epithelial-mesenchymal transition, and PI3K-AKT pathway-related genes. **Results:** Network pharmacology and molecular docking predicted AS-IV anti-glioma targets were associated with the PI3K/AKT pathway. AS-IV inhibited A172 and U251 cell proliferation and invasion dose- and time-dependently. It arrested the cell cycle in G0/G1 phase and induced apoptosis. AS-IV upregulated P21, Bax, and E-cadherin expression while downregulating CDK4, CDK6, Bcl-2, Vimentin, P-PI3K, and P-AKT. **Conclusion:** AS-IV exhibits anti-tumor effects against glioma cells, potentially by inhibiting the PI3K/AKT signaling pathway. This leads to apoptosis induction, proliferation suppression, cell cycle arrest, and invasion inhibition.

Key Words Astragaloside IV, Glioma, PI3K/AKT Pathway, Molecular Docking

INTRODUCTION

Glioma is the most common primary brain tumor, originating from glial cells or supporting cells in the central nervous system, accounting for approximately 80% of primary brain malignancies [1]. According to the morphological and malignant behavior classification of I-IV in WHO classification system, grade IV glioblastoma (GBM) has the highest degree of malignancy based on its morphological and malignant behavior classification of I–IV [1,2]. Due to its high invasiveness and resistance to treatment, glioma has a poor prognosis with high recurrence and mortality rates, significantly reducing patients' quality of life and cognitive abilities. Gliomas are among the most frequent primary brain tumors in the central nervous system. Because of its high level of invasion, the median survival time is typically shorter than one and a half years from diagnosis. This high case fatality rate could be attributed to the fact that surgery cannot completely remove the tumor without injuring the healthy brain. The survival rate of patients with glioblastoma increased, most likely as a result of temozolomide treatment [3]. However, chemotherapy must penetrate the blood-brain barrier and drug resistance, necessitating the discovery of new possible therapeutic medicines for clinical trials [4,5]. Traditional Chinese medicine provides distinct advantages in regulating the progression of patients' illnesses, minimizing recurrence, relieving symptoms and signs, increasing quality of life, and extending survival. In recent years, with the advancement of molecular biology, cell biology, genetics, and other sciences, research on the mechanism of traditional Chinese medicine in preventing and treating glioma has advanced to the micro-level of gene and cell signal transduction.

Astragalus membranaceus is a traditional Chinese medicine derived from the dried roots of leguminous plants

that has shown anti-inflammatory, anti-immune, anti-tumor, diabetic, and cardiovascular disease preventative qualities in modern pharmacological therapies [6,7], and it is also commonly used in Chinese medicine decoctions, particularly for cancer treatment [8-10], and its constituents have been employed in cancer treatment [10,11]. According to Chinese medicine' s theory, Astragalus membranaceus could balance qi and yang, stimulate diuresis and detumescence, firms the skin, and reduces perspiration [1,6]. Previous research has shown that Astragalus membranaceus could affect the occurrence and development of glioma cells by inhibiting their proliferation and inducing their apoptosis. Astragaloside IV, a major component of Astragalus membranaceus, shows enhanced efficacy when combined with cisplatin in treating C6 glioma compared to either agent alone. The mechanism is that a combination of drugs can increase pro-apoptotic gene expression while decreasing anti-apoptotic gene expression [8]. The active compounds, isoflavanone and 1,7-Dihydroxy-3,9-dimethoxy pterocarpene from Astragalus membranaceus have a multicomponent and multitarget synergistic therapeutic effect on glioblastoma by actively targeting numerous targets in various pathways [8]. Network pharmacology combing with experimental verification have become an effective and popular way in the application of Chinese herbs or its ingredients [12,13]. Previous research showed that the Potential Pharmacological Mechanism of Astragalus membranaceus on glioma using network pharmacology analysis, however, it is pity that this study was completed without experimental validation [8]. Therefore, this study was mainly designed to explore the underlying mechanism of Astragaloside IV's anti-glioma effects by detecting targets and signaling pathway predicted using network pharmacology and molecular. By investigating the mechanism of Astragaloside IV's anti-glioma action at the molecular level, this study would provide a theoretical and experimental basis for clinical application.

MATERIALS AND METHODS

Mechanism Prediction based on Network Pharmacology

The SwissTarget database[1] , TargetNet database[2] , Super-PRED database[3] and the pharmMapper database[4] were utilized to search for the target of pharmacological action of astragaloside IV and removes duplicate genes. The OMIM database[5], GeneCards database[6] , and TTD database[7] were used to screen the gene targets associated with glioma, and a disease target dataset was established. For the simplicity of unified analysis, all the following targets are available through the UniProt database[8] were converted to the UniProt ID format. The Venny Visual Analysis Platform[9] was used to create a Venn diagram of astragaloside IV and glioma targets. The STRING database[10] , PDB database[11] , and PubChem database[12] , and Cytoscape 3.8.2 software was used to visualize the possible target of astragaloside IV against glioma, which they both share. The Metascape's Database[13] was used to generate a KEGG pathway enrichment analysis on putative astragaloside IV targets in glioma treatment to better understand the role of relevant targets. The enrichment analysis results were visualized using WeChat, an online sketching platform[14] . This study also utilized astragaloside IV to treat glioma cells in vitro and investigates the goal of network pharmacology prediction. An online CB-Dock2 tool[15] [14] to perform molecular docking and visual presentation.

Materials and Reagents

This study utilized astragaloside IV to treat glioma cells in vitro and investigated the goal using network pharmacology prediction. Following four hours of incubation, the supernatant was removed, and each well received 150 µL of DMSO, which was shaken in the dark for ten minutes. The optical density (OD) at 490 nm was determined using an enzyme-linked immunosorbent assay (Molecular Devices, USA, model SpectraMax i3X). Each well had six multiple wells replicates, and the experiment was run for three times.

Cell Lines and Cell Culture

Human glioma cells A172 and U251 were cultured in high glucose DMEM medium (containing 10% Fetal bovine serum, penicillin 100 IU/mL, Streptomycin 100 µg/mL, placed in a constant temperature incubator at 37 ℃ and 5% CO_2.

MTT Experiment

This study utilized astragaloside IV to treat glioma cells in vitro and investigated the goal using network pharmacology prediction. Following four hours of incubation, the supernatant was removed, and each well received 150 µL of DMSO, which was shaken in the dark for ten minutes. The optical density (OD) at 490 nm was determined using an enzyme-linked immunosorbent assay (Molecular Devices, USA, model SpectraMax i3X). Each well had six multiple wells replicates, and the experiment was run for three times.

Cell Scratch Healing Test to Detect Cell Migration Ability

Using a marker pen and a ruler, a straight line was first drawn on the back of the six-well plate at intervals of one centimeter. After extracting the cell suspension from each well, the plate with six wells was used. Four groups were

[1] http://swisstargetprediction.ch/
[2] http://targetnet.scbdd.com/calcnet/index/
[3] https://prediction.charite.de/index.php
[4] www.lilab ecust.cn/sharmmapper/
[5] https://www.omim.org/
[6] https://www.genecards.org/
[7] http://db.idrblab.net/ttd
[8] https://www.uniprot.org/
[9] https://bioinfogp.cnb.csic.es/tools/venny/
[10] https://string-db.org/
[11] https://www.rcsb.org/
[12] https://pubchem.ncbi.nlm.nih.gov/
[13] https://metascape.org/
[14] http://www.bioinformatics.com.cn
[15] https://cadd.labshare.cn/cb-dock2/php/index.php

included in this experiment: the control, astragaloside IV with low, medium, and high dosage groups. Every group had three multiple wells, and the cultures were kept in an incubator at 37°C. The culture media will be taken out of the six-well plate once the cell density reach 100% confluent and the line will be vertically scratched on the cell surface. The cells will be gently washed three times using 2 mL of PBS poured along the well wall. A volume of 2 mL of serum-free culture material was added, and zero-hour photos were taken with a microscope. After 48 hours of incubation, the cells were harvested and inspected under a microscope. The cell culture medium was altered to allow for 48 hours of photography. The scratch area was calculated using ImageJ software with the following formula: cell scratch healing rate = (cell scratch area (0h) - cell scratch area (48h))/cell scratch area (0h). The experiment was conducted for three times.

Transwell Detection of Cell Invasiveness

The Matrigel base adhesive was removed from the -20°C refrigerator in advance to 4°C to dissolve overnight. Before the experiment, pre-cool the pipette and culture medium at 4°C for about 2 hours. The matrix gel with serum-free medium was diluted at a dilution ratio of 1:8 (matrix gel with serum-free medium), blown, mixed, and mixed well, and put on the 24-hole plate on ice. The experiment was divided into four groups, namely the control group, low, medium, and high-dose groups. Each group was set with three wells, and the prepared Matrigel gel was added to the six-well plate. The plate was incubated at 37°C for 30 minutes. An appropriate amount of complete culture medium was added, blown, and mixed well, and its concentration was adjusted to 5×10^5/mL. A 200 μL serum-free cell culture was placed in a tiny chamber coated with Matrigel adhesive. The lower compartment was filled with 500 μL of full culture media and cultured for 48 hours in an incubator. The growing media in the chamber was removed, and an adequate volume of precooled PBS was added to wash the cells twice. The cells were then fixed at the lower side of the chamber for 2 minutes with 4% paraformaldehyde. The fixative was poured out, rinsed twice with PBS, then fixed with 100% methanol. The methanol was drained after 25 minutes and washed twice with enough PBS. After adding the 0.1% crystal violet, they were dyed in the dark for 20 minutes. After emptying the crystal violet dye, enough PBS was used to wash it twice. After that, the interior side of the chamber's cells were meticulously wiped with a cotton swab and let to remain at room temperature for around half an hour. Five visual fields in the center were randomly selected for photography, and the number of transferred cells in each group was calculated using Image J software.

Cell Cycle Detection by Flow Cytometry

After treating cells with 25% trypsin and washing twice with precooled PBS, the supernatant was collected. 1 mL of precooled 70% ethanol was added, gently blown and mixed, and fixed at 4°C for over 12 hours. The samples were centrifuged at 1000 r/min for 5 minutes and rinsed twice with precooled PBS. After adding PI and RnaseA, the samples were placed in a dark temperature bath at 37°C for 30 minutes before detection with flow cytometry. The experiment was conducted three times.

Cell Cycle Detection by Flow Apoptosis

The cells were gently digested and collected with 25% trypsin, washed twice with PBS, and the supernatant discarded. To start, cells were resuspended in 1 × Binding Buffer. In each tube, 5 μL of AnnexinV-APC and 10 μL of 7-AAD was added, mixed carefully, and incubated for 15 minutes at room temperature in the dark without washing. Then, each tube received 380 μL of precooled 1 × Binding Buffer. The apoptosis rate was determined using flow cytometry (BD, model FACSVerse, USA), and the procedure was repeated three time for statistically analysed.

Western Blotting

The cultured cells were harvested and lysed on ice with RIPA buffer containing protease inhibitors for 30 minutes. The proteins were then separated by SDS-PAGE and transferred to a PVDF membrane. After that, the membrane was sealed at room temperature with 5% skim milk powder for 1 hour and incubated overnight with the first antibody (1:1000) at 4 ℃. Following washing with 0.5% TBST, the membrane was incubated with the second antibody (1:2500) coupled with Horseradish peroxidase (HRP), and ECL reagent was used to visualize protein band signals.

Statistical Methods

The experimental data is expressed as mean ± standard deviation using SPSS 23.0 statistical software was used for statistical processing, and the differences between the two groups were analyzed through Student's t-test. One way ANOVA was used for comparison between multiple groups, and the difference was statistically significant when $P < 0.05$.

RESULTS

Network Pharmacology Prediction and Molecular Docking

The targets for astragaloside IV intervention in glioma based on different databases of "drug targets" were screened; a total of 475 targets were obtained based on targetNET, 100 targets were obtained based on SWISS, 180 targets were obtained based on superPREDT, 291 targets were obtained based on pharmmapper, and 762 "drug targets" were obtained after deduplication. Based on the "disease target" screening, a total of 5311 glioma targets were identified. Intersecting the target of astragaloside IV with the target of glioma, a total of 494 targets were obtained, as shown in Figure 1A. The "astragaloside pathway target glioma" network diagram was drawn using Cytoscape 3.8.0 for 494 potential targets, as shown in Figure 1B. The interaction relationships between the core genes of the drug targets were shown in Figure 1A,B,C,D.

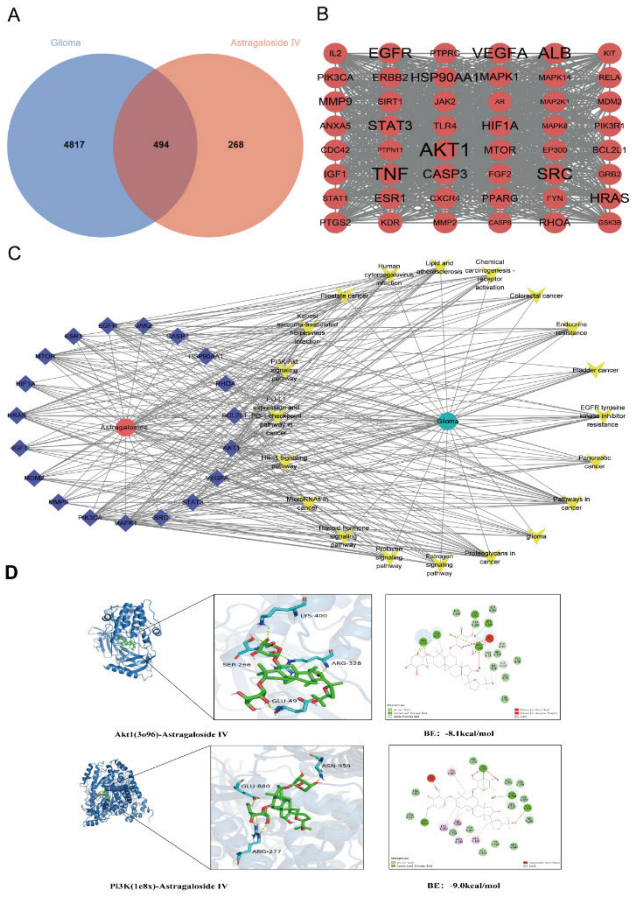

Figure 1: Network pharmocology and molecular docking results

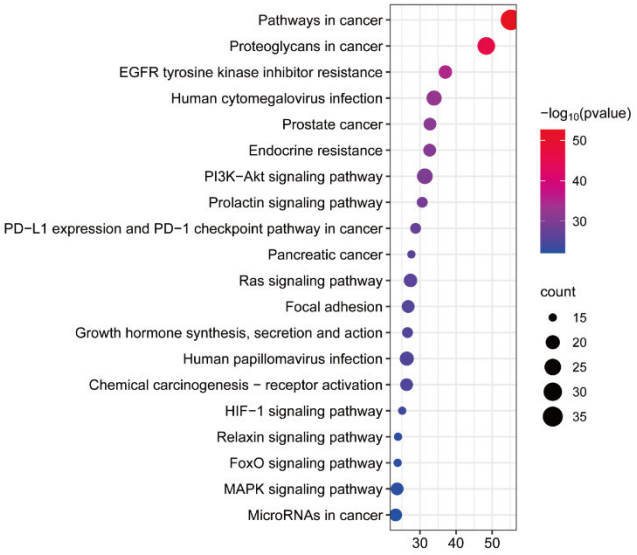

Figure 2: KEGG analysis results

Potential targets of Astragaloside IV (AS-IV) against glioma were identified by integrating drug target databases (TargetNET: 475 targets, SwissTarget: 100, SuperPred: 180, PharmMapper: 291), yielding 762 unique drug targets after deduplication. Glioma-related targets totaled 5,311 from disease databases. Intersection analysis revealed 494 overlapping targets implicated in AS-IV's anti-glioma effects (Fig. 1A). A compound-target-pathway-glioma interaction network for these 494 targets was visualized using Cytoscape (Fig. 1B), with core gene interactions mapped separately (Fig. 1C). Astroloside IV showed strong binding to key proteins AKT1 and PI3K, with binding energies of -8.1 kcal/mol and -9.0 kcal/mol respectively. With AKT1 (PDB:1e8x), it formed hydrogen bonds with ASN959, ARG277, GLU880, and GLN291; hydrophobic interactions with LYS883, LEU865, VAL882, ARG849, and PRO866; and an unfavorable donor interaction with SER955. For PI3K (PDB:3o96), hydrogen bonds occurred with GLU49, ALA50, LYS400, SER266, and ARG328, while van der Waals interactions involved 12 residues (e.g., HIS265, ASP398, TYR38), plus an unfavorable donor interaction with SER396 (Fig. 1D).

KEGG Pathway Enrichment Analysis

The potential targets of astragaloside IV for anti-glioma were imported into the Metascape database. The analysis results were downloaded and saved in CSV format once the operation was finished. After that, the result file was opened, and the log (q value) was changed to a q value using the POWER function. The findings were ordered from small to large and the screening was based on $q < 0.001$. The top 20 pathways were selected for visualization. Among the first 20 KEGG signaling pathways enriched by potential anti-glioma targets of astragaloside IV, the tumor-related signaling pathways mainly included: cancer pathway, Proteoglycan in cancer, resistance to EGFR Tyrosine kinase inhibitor, Human betaherpesvirus 5 infection, prostate cancer, endocrine resistance, PI3K-Akt signaling pathway, PD-L1 in cancer and PD-1 checkpoint pathway, pancreatic cancer, Ras signaling pathway, attachment spot Synthesis, secretion and function of growth hormone, human papillomavirus infection, chemical carcinogenesis receptor activation, HIF-1 signaling pathway, Relaxin signaling pathway, MAPK signaling pathway microRNAs in cancer. Based on the network pharmacology prediction results, some potential targets of astragaloside IV against glioma are related to the PI3K-AKT signaling pathway. Therefore, in light of literature reports on the expression of PI3K-AKT in gliomas, we have selected the PI3K/AKT pathway for experimental validation, as shown in Figure 2.

Analysis of the top 20 enriched KEGG pathways revealed AS-IV potentially targets glioma through cancer-related pathways including PI3K-AKT, MAPK, RAS, PD-1/PD-L1, HIF-1, and microRNAs in cancer. Given the PI3K-AKT pathway's established role in glioma per literature and its prominence in network pharmacology predictions, this pathway was prioritized for experimental validation (Fig. 2).

The Effect of Astragaloside IV on the Proliferation of Glioma Cells

To investigate the effect of astragaloside IV on cell proliferation in glioma cells, MTT experiments were used to

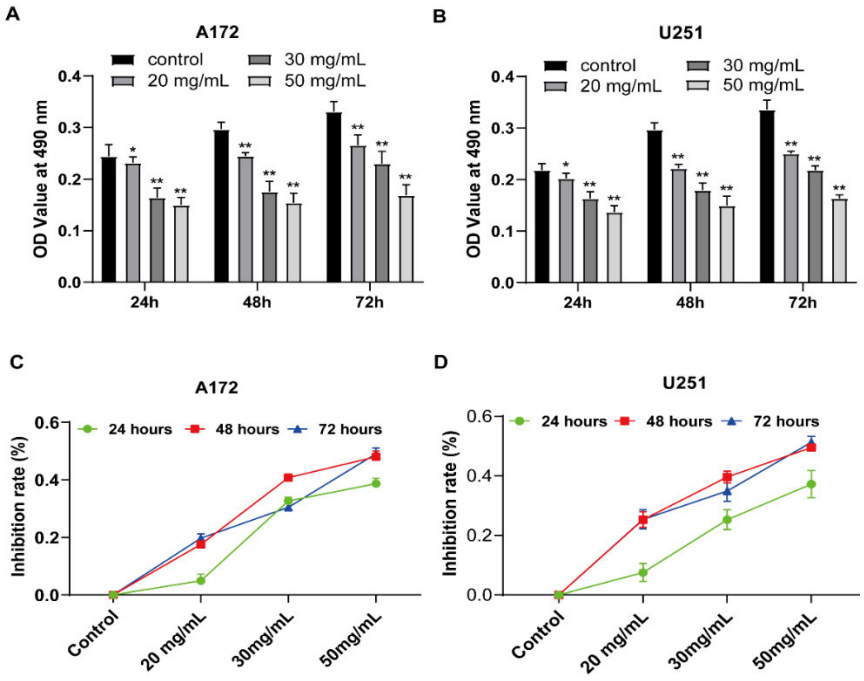

Figure3: The effect of AS-IV on the proliferation of glioma cells

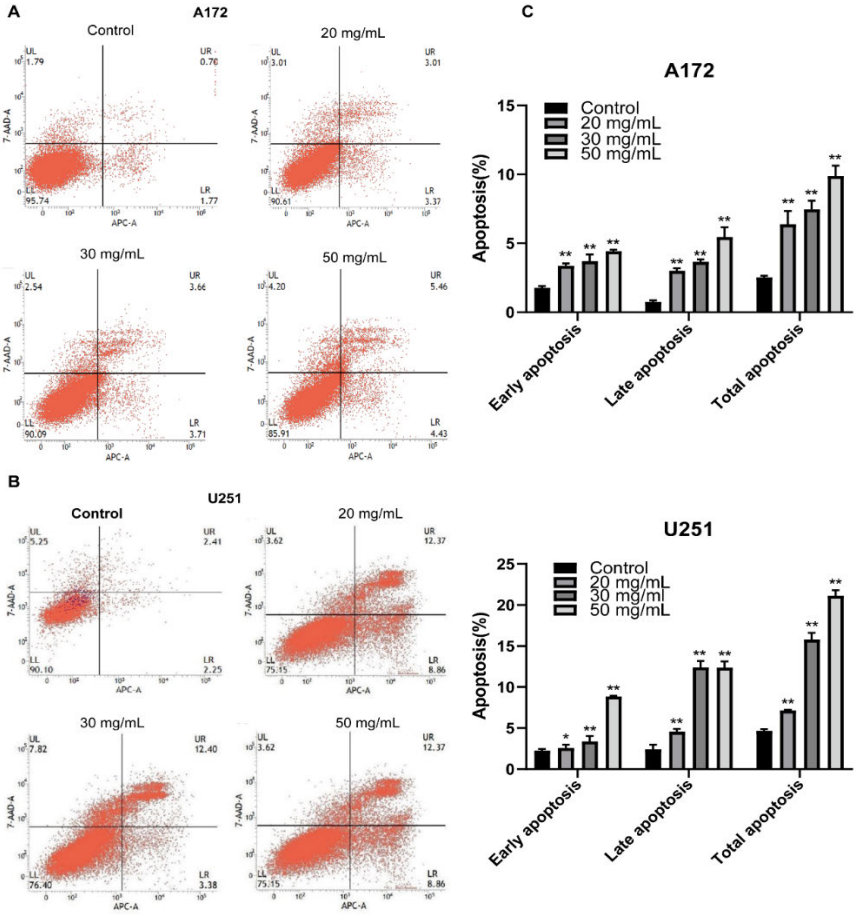

Figure 4: The effect of AS-IV on inducing apoptosis of glioma cell

detect the OD values of different concentrations of the treated group and the control group. The OD values were measured at three time periods: 24 hours, 48 hours, and 72 hours. The results showed that drug concentrations of 20, 30, and 50 mg/mL could inhibit the proliferation of glioma cells in a time- and dose-dependent manner with statistical significance ($p<0.05$), as shown in Figure3A,B,C,D.

MTT assays demonstrated that AS-IV significantly inhibits glioma cell proliferation in a time- and dose-dependent manner. Treatment with 20, 30, and 50 mg/mL concentrations reduced cell viability at 24h, 48h, and 72h (all $p<0.05$), as quantified through OD measurements (Figure 3A-D).

The Effect of Astragaloside IV on the Cell Cycle of Glioma Cells

To investigate the effect of astragaloside IV on the cell cycle of glioma cells, flow cytometry was used to detect the cycle changes of glioma cells under different drug concentrations. The results showed that the data analysis showed statistical significance, as shown in Figure 4A,B,C,D. This result suggests that astragaloside IV may cause glioma cells to enter the G0/G1 phase and become stagnant.

Flow cytometry analysis revealed that AS-IV dose-dependently induces G0/G1 phase arrest in glioma cells (Figure 4A-D), showing statistically significant cell cycle alterations ($p<0.05$). This suggests the drug inhibits glioma progression by blocking cell cycle progression at the G0/G1 checkpoint.

The Effect of Astragaloside IV on Inducing Apoptosis of Glioma Cells

In order to examine astragaloside IV's impact on glioma cell apoptosis in more detail, cells treated with varying doses (20, 30, and 50 μg/mL) for 48 hours were identified using flow cytometry. The results showed that the astragaloside IV can induce apoptosis of glioma cells in a dose-dependent manner, as shown in Figure 5A,B,C.

The Effect of Astragaloside IV on the Lateral Migration and Invasion Ability of Glioma Cell Scratch Healing

The cells treated with the drug were subjected to scratch healing experiments and photographed under a microscope at 0 and 48 hours, respectively. The experimental results were statistically analyzed and quantified using ImageJ software. After 48 hours, the width of scratch healing in the treatment group was significantly wider than that in the control group. the scratch area was measured using the formula:

$$\text{Healing rate} = \frac{\text{Cell scratch area (0h)} - \text{Cell scratch area (48h)}}{\text{Cell scratch area (0h)}} \times 100\%$$

The results showed that compared with the control group, the scratch healing rate of the two cell lines in the treatment group was significantly reduced ($p<0.01$). This indicates that astragaloside IV could inhibit the migration ability of glioma cells.

The Transwell experiment was used to detect the effect of astragaloside IV on the invasion ability of glioma cells. After 48 hours, photos were taken and the number of transmembrane cells was calculated using ImageJ software. A172 and U251 showed the same results, with a significant decrease in the number of transmembrane penetrating cells in the treatment group compared to the control group ($p<0.01$). This indicates that astragaloside IV can inhibit the invasive ability of glioma cells, as shown in Figure 6A,B,C,D.

AS-IV significantly inhibited glioma cell migration and invasion in vitro. Scratch assays revealed reduced healing rates in treated cells (vs. controls, $p<0.01$) after 48 hours, indicating impaired migration. Transwell experiments similarly showed decreased invasive capacity, with fewer transmembrane cells in A172 and U251 lines ($p<0.01$). Both assays, quantified using ImageJ and illustrated in Figure 6A-D, demonstrate AS-IV 's potent anti-metastatic effects on glioma cells.

The Effects of Astragaloside IV on the Expression of Genes related to Cell Cycle, Apoptosis, Invasion, and Migration

To investigate the specific mechanism of astragaloside IV inhibiting cells in G0/G1 phase, western blotting was performed to assess the expression of G0/G1 phase and apoptosis-related genes. The results showed that following administration, the expression of CDK4 and CDK6, which regulate the G0/G1 phase, reduced, while P21 increased. The expression of pro-apoptotic gene Bax increased, while anti-apoptotic gene Bcl-2 decreased (both $p<0.01$). In conclusion, these findings indicate that astragaloside IV may limit cell growth by inhibiting cells in the G0/G1 phase and triggering apoptosis. Epithelial-mesenchymal transition (EMT) is an important mechanism that promotes the invasion and migration of glioma cells. Therefore, blocking the EMT process can inhibit cell invasion and migration [15]. In order to investigate whether the inhibitory effect of astragaloside IV on cell invasion and migration is also caused by reducing the occurrence of EMT, western blot was used to detect the expressions of EMT related targets, such as E-cadherin and Vimentin. Compared with the control group, the expression of E-cadherin increased in the treatment group, while the expression of Vimentin decreased (all $p<0.01$). The above findings suggest that astragaloside IV may reduce the invasion and migration ability of glioma cells by inhibiting EMT, as shown in Figure 7A,B.

Western blot analysis revealed AS-IV: (1) induced G0/G1 arrest (↓CDK4/6, ↑p21); (2) promoted apoptosis (↑Bax, ↓Bcl-2, $P<0.01$); and (3) inhibited EMT (↑E-cadherin, ↓Vimentin, $P<0.01$). These findings demonstrate multi-targeted anti-glioma effects through cell cycle blockade, apoptosis induction, and metastasis suppression (Figure 7 A, B).

The Effects of Astragaloside IV on the PI3K-AKT Signaling Pathway

To investigate the relationship between astragaloside IV and the PI3K-AKT pathway in glioma cells, genes associated with this pathway were selected for detection by western blot. In both cell lines, the treatment group showed lower expression of PI3K, AKT, P-PI3K, and P-AKT compared to

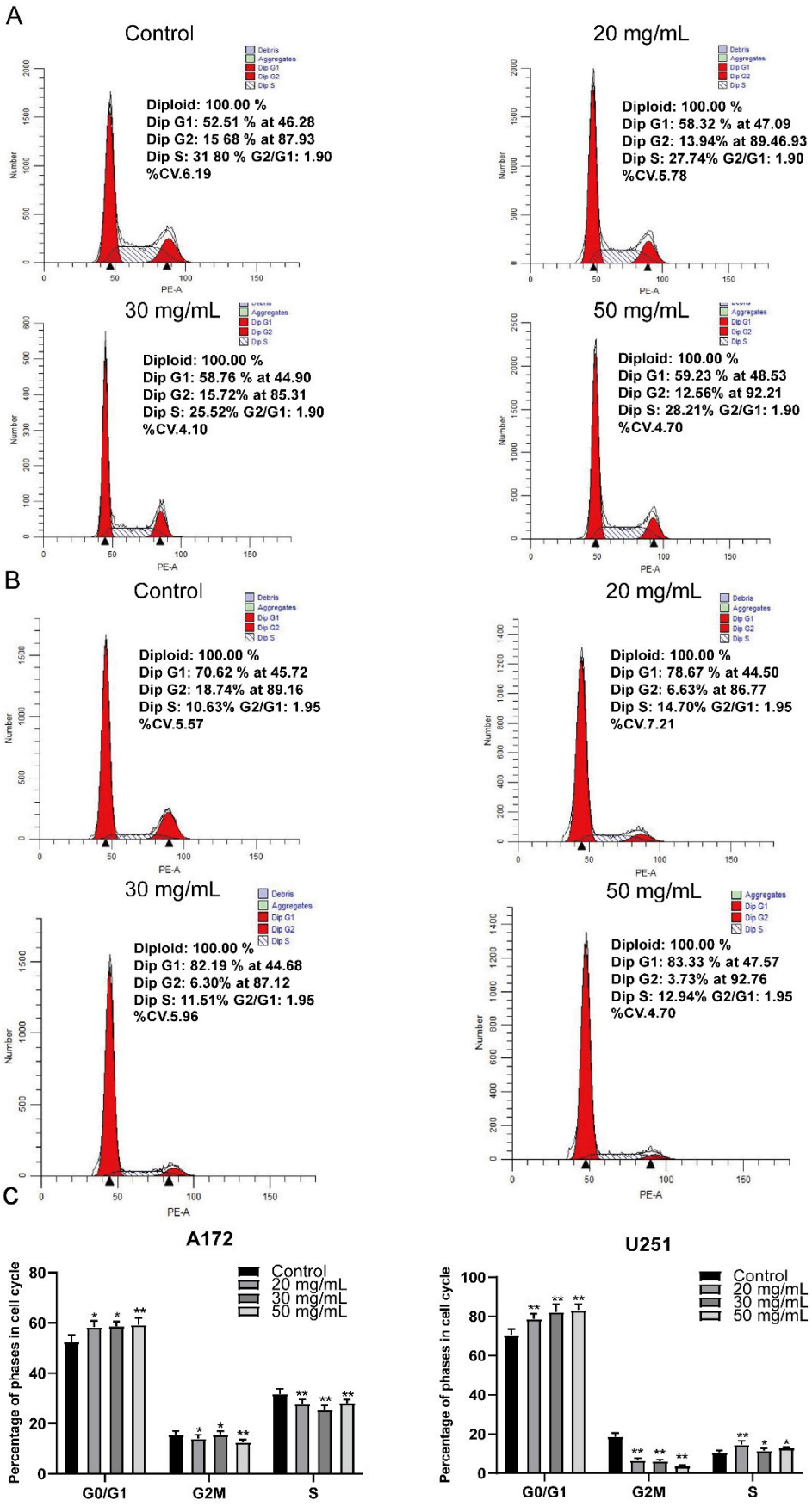

Figure 5: The effect of AS-IV on the cell cycle of glioma cells

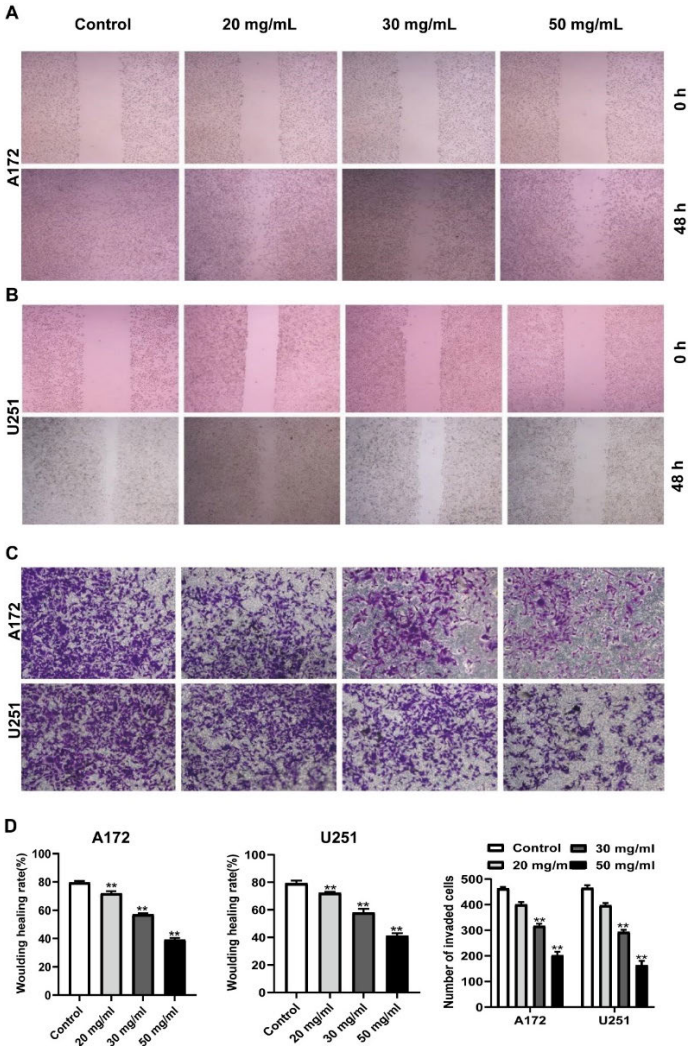

Figure 6: The effect of AS-IV on migration and invasion ability of glioma cell scratch healing

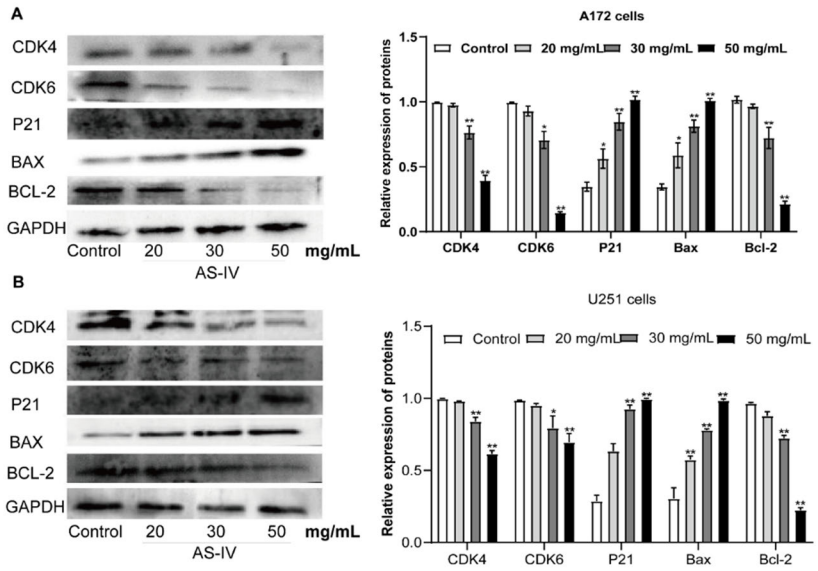

Figure 7: The effects of AS-IV on the expression of genes related to cell cycle, apoptosis, invasion, and migration

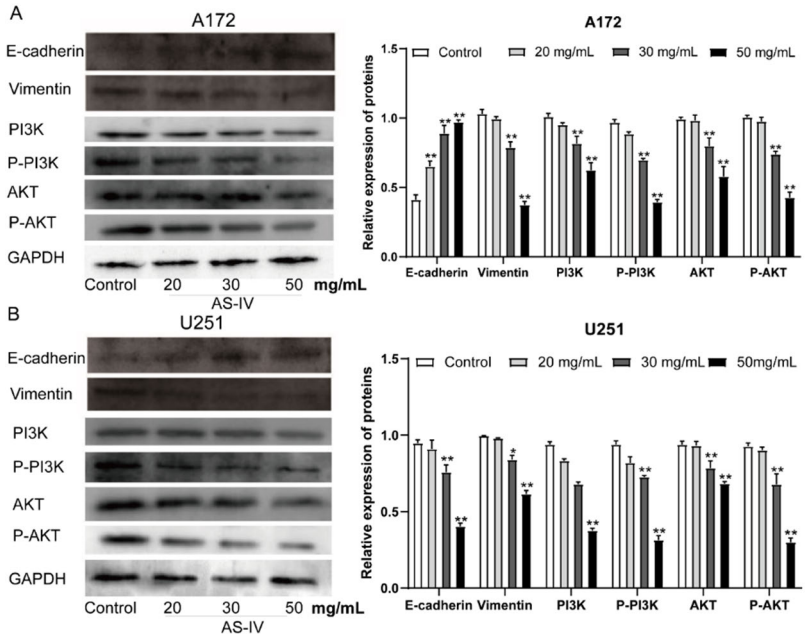

Figure 8: The effects of AS-IV on the AKT-PI3K signaling pathway

the control group ($p<0.05$). The data above show that astragaloside IV could inhibit the PI3K-AKT pathway in glioma cells, as shown in Figure 8A,B.

The AS-IV treatment group exhibited significantly lower expression levels of PI3K, AKT, p-PI3K, and p-AKT compared with the control group ($p<0.05$). The data above show that AS-IV could inhibit the PI3K-AKT pathway in glioma cells, as shown in Figure 8A,B.

DISCUSSION

Gliomas account for approximately 47.1% of all malignant tumors in the central nervous system and are considered the deadliest primary brain tumor [7]. Despite recent progress in surgery, chemotherapy, and radiation therapy, the prognosis of glioma has not significantly improved, and the overall survival rate is low [8]. Therefore, the auxiliary treatment of traditional Chinese medicine is expected to become an effective treatment strategy for glioma. Currently, research has found that astragaloside IV plays a key role in some non-tumor diseases, such as in regulating TLR4/MyD88/NF- κB, and the activity of which signal transduction reduces inflammation and oxidative stress responses, thereby alleviating PM2.5-induced lung injury [16]. Furthermore, by changing microglia from an inflammatory M1 to an anti-inflammatory M2 phenotype, astragaloside IV may suppress the TLR4/NF-κB signaling pathway, have an anti-inflammatory effect on microglia, and shield neurons from microglia-mediated cell death [16,17]. Astragaloside IV plays a therapeutic role in ARDS by improving the tight junction and cell vitality of the LPS-stimulated pulmonary endothelial ARDS cell model and lowering cell apoptosis. It can also directly or indirectly inhibit the occurrence of autophagy by inhibiting oxidative stress and inflammatory reactions [18]. Astragaloside IV may partially confer neuroprotective benefits against transient cerebral ischemia and reperfusion by blocking the TLR4 signaling pathway and excessively activating the NLRP3 inflammasome [19]. Astragaloside IV could exhibit noteworthy properties in restraining the growth and spread of diverse cancers, including ovarian, lung, and liver cancers, and it has the potential to serve as an anti-tumor medication [20].

Based on network pharmacology prediction analysis, a total of 494 putative targets of astragalus membranaceus and astragaloside IV were evaluated for their anti-glioma properties in this study. The KEGG pathway enrichment study indicated that some of the targets were enriched in PI3K-AKT signaling pathway. The PI3K-AKT signaling pathway was considered as one of the essential pathways that govern cell cycle, apoptosis, oxidative stress, DNA damage repair, proliferation, and a critical regulator of epithelial-mesenchymal transition [21,22], and the inhibitors of this pathway were believed as therapeutic target [21]. In order to investigate the impacts on glioma cell lines' proliferation, cycle, and apoptosis [23,24]. we attempted to utilize varying amounts of astragaloside IV to disrupt the cells. The findings demonstrated that astragaloside IV exhibited a noteworthy inhibitory impact on glioma cells at drug doses of 20 μg/mL, 30 μg/mL, and 50 μg/mL. These three medication concentrations were so chosen for further studies.

Astragaloside IV could suppress invasion, and migration greatly, as demonstrated by cell scratch, and transwell assays. Tumor infiltration and distant metastases are significantly initiated from EMT, and which determins distant metastasis through its biomarkers [18]. In order to investigate the possibility that astragaloside IV may inhibit glioma cell invasion and migration by affecting EMT, we used western blot to measure the expression of the vimentin and E-cadherin genes. Astragaloside IV was shown to

restrict the invasion and migration capacities of glioma cells by upregulating the expression of E-cadherin, a target relevant to epithelial-mesenchymal transition, and downregulating the expression of vimentin. The transmembrane glycoprotein E-Cadherin belongs to the family of adhesion molecules that are reliant on calcium and found in epithelial cells [25]. It is vital for tissue growth and development. Its function can be reduced or destroyed, causing cell connections to break down and malignant cells to enter and spread. In cancer, E-cadherin expression correlates with tumor differentiation. E-Cadherin showed an inverse correlation with tumor differentiation, lymph node metastases, and mesangial tumor deposits. E-Cadherin was correlated inversely with tumor differentiation, lymph node metastases and mesangial tumor deposits [26]. Vimentin is a highly dynamic and adaptable cytoskeletal protein that has been found to be involved in a number of critical wound healing processes, including the epithelium-mesenchymal transition (EMT), which increases the motility and dynamic properties of epithelial cells during wound healing and metastasis [27]. In cancer, overexpression of vimentin is positively correlated with tumor invasion and growth at an accelerated rate as well as a poor prognosis. Vimentin has gained recognition as a marker for the epithelial-mesenchymal transition (EMT) in recent years. Collectively, Vimentin is a desirable prospective target for anti-cancer treatment [28].

In this study, Astragaloside IV inhibited cells in the G1/G0 phase and trigger apoptosis in glioma cells, as demonstrated by MTT. Western blotting experiment showed that the expression of associated genes CDK4 and CDK6 was dramatically reduced in the G1/G0 phase, while the expression of P21 increased significantly. The expression of proapoptotic gene Bax was increased, while the expression of tumor suppressor gene Bcl-2 was decreased. The cell cycle machinery that drives the G1 to S phase transition by phosphorylating and inactivating the retinoblastoma proteins mostly dependent on D-type cyclins, CDK4, and CDK6. Numerous pieces of data demonstrate the pivotal function of the cyclin Ds-CDKs axis in cancer via modulating apoptosis, angiogenesis, migration, senescence, and proliferation [29]. The majority of other tumor types have only seen minimal efficacy with CDK4/6 inhibitors; however, they are beneficial in treating metastatic breast cancer. It is demonstrated here that cancers with low CDK6 expression are highly responsive to CDK4/6i and depend on CDK4 function overall [30]. In a number of cancer forms, constitutive activation of cyclin D–CDK4/6 is the primary cause of carcinogenesis. Several different tumor types are undergoing clinical trials for small-molecule CDK4/6 inhibitors, which have been utilized to great effect in the treatment of hormone receptor-positive breast tumors [29].

Previous research indicated that astragaloside IV could play antitumor effects via Wnt/AKT/GSK-3β/β-catenin, TGF-β/PI3K/AKT/mTOR, PI3K/MAPK/mTOR, PI3K/AKT/NF-κB, Rac family small GTPase1/RAS/MAPK/ERK, TNF-α/protein kinase C/ERK1/2/NF-κB and Tregs/IL-11/STAT3 signaling pathways by in vitro and in vivo studies [30]. It appears that a pivotal role for the PI3K/AKT pathway plays in them. Combing with network pharmacology prediction, this study used western blotting to determine if astragaloside IV could alter glioma growth by activating the PI3K-AKT pathway. The results showed that Astragaloside IV could reduce the expressions of PI3K, AKT, P-PI3K, and P-AKT protein levels dramatically. This also suggests that astragaloside IV's reduction of glioma cell proliferation, invasion, and migration could be linked to the inactivation of the PI3K-AKT pathway, which is compatible with the prediction result of network pharmacology.

CONCLUSIONS

To summarize, astragaloside IV inhibits glioma cell proliferation, invasion, and migration via the PI3K-AKT signaling pathway. Simultaneously, this study represents the characteristics of Astragalus membranaceus multitarget action, which is critical for understanding the mechanism of Astragalus membranaceus anti-glioma.

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